Mary E. Kicza, Associate Administrator
NASA Headquarters, Code U
300 E. Street SW
Washington, D.C. 20546

October 17, 2003

Dear Ms. Kicza,

We wish to call attention to an important area of research, the fundamental science of protein crystal growth, that is yielding deep scientific insights into protein crystallization, protein behavior in complex fluids, and related problems, and that will have a major impact on progress and practice in structural biology in the future. It is an area that has been nurtured for some 20 years by NASA research support and is especially noteworthy for the high quality of its peer-reviewed scientific research and the excellence of its publication record. This fundamental research impacts phase transformation kinetics in general, and has dramatically increased our understanding of protein crystallization in particular. It is an area whose continued progress is now threatened by budgetary and programmatic changes, however, its remarkable record of productivity and the extraordinary quality of its scientific contributions argue strongly for extraordinary measures to assure its continued support.

Inspired by NASA's observation that some protein and virus crystals grow with improved quality in microgravity, this ground-based research program has illuminated the fundamental physical principles governing protein crystallization and their relation to those for small molecules and inorganic materials. More broadly, it has helped define the series of complex phase transformation problems of the crystallization process that lie between purification of a protein sample and crystallographic data collection and that, in this era of high-throughput structural genomics, have become even more obviously the major bottleneck to structure determination. This research provides a solid foundation in materials science for the rational design of crystallization experiments and of pre- and post-crystallization protocols, and has led to the development of new diagnostic tools for use in the crystallization laboratory. It will impact not only macromolecular structure determination and drug discovery, but also the development of crystal-based biological catalysts, the formulation and delivery of protein-based drugs, and the long-term storage of biological macromolecules, all of which have important consequences for long-term human habitation in space.

Protein crystals are of interest for fundamental studies because they are intermediate between conventional inorganic and small molecule crystals, colloidal crystals, and solid polymers. Like conventional crystals they often grow with excellent long-range order and low mosaicity. Like colloidal crystals, the crystallizing molecules are large compared with the range of interactions between molecules. Like solid molecular polymers, the individual macromolecules have extensive conformational flexibility, which can destroy short-range order and diffraction resolution. The unique features of protein crystals include the weakness and complexity of their intermolecular interactions, which lead to slow growth rates, the ability to modify intermolecular interactions through solution chemistry and genetic manipulation, the large role played by

impurities and microheterogeneities, their large solvent content and fundamentally composite nature, and their exceptional softness and fragility.

NASA's program of peer-reviewed support has been responsible for almost every important contribution to understanding of the fundamentals of protein solutions and protein crystallization in recent years. The breakthroughs produced by NASA's support include establishment of the correlation between the second virial coefficient characterizing protein-protein interactions and the formation of crystals, which has provided an initial assay for crystallization conditions with dilute protein solutions [Wilson, Lenhoff]; the extension of this approach to membrane proteins [Loll, Wiencek]; the quantitative understanding of the complex phase diagrams for protein solutions (which often include high and low-density liquid phases as well as crystalline and gel phases) and, importantly, the recognition of the role of the liquid-liquid phase separation in crystal nucleation [Benedek, Fraden, Zukoski]; direct observation of protein nucleation events and an explanation of the nucleation mechanism [Vekilov]; quantitative determination of fundamental nucleation and growth parameters [McPherson, Malkin, DeYoreo, Vekilov]; direct observation by atomic force microscopy (AFM) of growing protein crystals at molecular resolution, which has led to elucidation of crystal growth mechanisms and crystal defects for a variety of soluble and membrane proteins and for viruses [DeYoreo, McPherson, Malkin]; identification of the mechanism of protein crystal growth cessation, which limits the size of protein crystals and is one of the most important practical problems faced by crystallographers [Malkin, Vekilov]; identification and quantification of effects of impurities in growth and crystal disorder, including the purifying effect of the microgravity environment, and direct 3D visualization of impurity incorporation and defect formation [Chernov, Vekilov, Thomas, Carter, Thorne]; introduction of a high-throughput, nanoliter system integrated with incomplete factorial and neural net capabilities for screening of crystallization conditions [DeLucas]; application of conventional and new far-field X-ray imaging techniques and high-resolution reciprocal space mapping to identify crystal defects, the character and origin of crystal mosaicity, and the relation between internal stress and diffraction quality [Thorne, Snell, Borgstahl, Hu, Chernov]; and determination of how cryoprotective procedures, which are used to reduce crystal damage by X-rays, themselves damage crystals (which also has implications for cryopreservation of cells and tissues) [Thorne, Snell, Borgstahl]. A list of selected references is appended.

This NASA-supported work has an international reputation, of course, and is augmented by a large number of studies outside the U.S. These are documented in, for example, the nine International Conferences on the Crystallization of Biological Macromolecules (ICCBM) held to date, as well as elsewhere in the scientific literature. This body of work provides a solid foundation from which to address important questions in the science of phase transformations in complex fluids and in the physics of solids comprised of nanoscale, anisotropic, and conformationally variable species.

The future impact of this NASA-supported work on structural biology will be significant. So far, the developments that have the greatest day-to-day impact have largely come from biology: genetic modifications to obtain molecular forms that may be easier to crystallize, improved purification methods based on expression tags, and crystal screens (arrays of premixed crystallization cocktails). With the advent of high-throughput methods, much purification and crystallization will be performed robotically, and detailed and voluminous data on the results of

crystallization experiments will be accessible for computer processing. This provides an ideal environment in which to implement algorithms based on insights from fundamental crystal growth studies. Even if the present structural genomics effort is successful beyond the most optimistic predictions, many difficult and important structures – including most membrane proteins (which provide the majority of drug targets) and most protein complexes – will remain to be studied. Structural biologists will demand higher resolution structures than they can now obtain and may need data collection at room temperature in order to fully analyze mechanisms of molecular function. They will want neutron diffraction studies to define more clearly the patterns of hydrogen bonding and the roles that solvent molecules play in the structure and function of specific proteins, studies made possible by new facilities and developments for neutron diffraction. These longer-term requirements can only be met by producing more perfect and, in some cases, much larger crystals, and this will require fundamental understanding of crystal growth.

It is widely recognized that NIH, NSF, and DOE, the major supporters of protein structure determination research in this country, have never really supported crystallization studies, not even for membrane proteins. The fundamental science of protein crystal growth is an area of important research that NASA, through its support alone, has brought into being. It is an area that, because of its quality and productivity, NASA can justifiably point to with pride. Its continued health is imperiled by abrupt and disruptive loss of support and NASA must act to avoid this catastrophe. Continuing support of the best peer-reviewed, science-oriented studies – ground-based and flight-based – will maintain progress in an area that NASA created, and will contribute both to NASA's manned exploration of space and to our nation's health and economic future.

Signed: _____

Print Name: _____

Date: _____

CC: E. Trinh, B. Carpenter, F. Chiaramonte, C. Clinton, S. Davison, M. King, C. Kundrot, M. Lee, M. Wargo

Attached: References

List of Signers

Anderson, Wayne
Molecular Pharmacology & Biological Chemistry, Northwestern University

Booth, Nicholas
Center for Microgravity & Materials Research
University of Alabama, Huntsville

Borgstahl, Gloria
Department of Chemistry
University of Toledo

Bugg, Charlie
BioCryst Pharmaceuticals, Inc.
Birmingham, Alabama

Bunick, Gerard
University of Tennessee

Carter, Dan
New Century Pharmaceuticals, Inc
Huntsville, Alabama

Carter, Charlie
University of North Carolina, Chapel Hill

Chayen, Naomi
Imperial College, London

Chernov, Alexander
Universities Space Research Association
Huntsville, AL

Dauter, Zbigniew
Macromolecular Crystallography Laboratory
National Cancer Institute

Deisenhofer, Johann
University of Texas Southwestern
Medical Center at Dallas

DeLucas, Larry
Biochemistry and Molecular Genetics
University of Alabama, Birmingham

Drenth, Jan
Laboratory of Biophysical Chemistry
The Netherlands

Duax, William
Hauptman-Woodward Med. Res. Inst.

Einspahr, Howard
Bristol-Myers Squibb (retired)

Erdmann, Volker
Free University Berlin
Institute for Chemistry / Biochemistry

Fraden, Seth
Physics Department
Brandeis University

Olga Gliko
University of Houston
Department of Chemical Engineering

Hauptmann, H.
Hauptman-Woodward
Medical Research Institute

Helliwell, John
Department of Chemistry
The University of Manchester, UK

Hu, Z. W.
NASA/Marshall Space Flight Center, AL

Jones, Noel
Eli Lilly (Retired)

Jurnak, Frances
Physiology and Biophysics
University of Califonia, Irvine

Karle, Jerome
Laboratory for the Structure of Matter
Naval Research Laboratory, Washington, D.C.

Komatsu, Hiroshi
Institute for Materials Research
Tohoku University, Sendai Japan

Lenhoff, Abraham
University of Delaware

Loll, Partick
MCP Hahnemann University, PA

Malkin, Alexander
Dept. Chemistry and Material Science
Lawrence Livermore Lab

McPherson, Alexander
Molecular Biology & Biochemistry
University of Califonia, Irvine

Muchmore, Steven
Abbott Laboratories, IL

Ocko, Benjamin
Physics Department
Brookhaven National Laboratory

Rupp, Bernard
Lawrence Livermore Natl. Laboratory

Santersiero, Bernard
Chicago Inst. of Technology

Snell, Edward
Universities Space Research Association
NASA Marshall Space Flight Center

Stallings, William
Pfizer, St. Louis

Sweet, Robert
Brookhaven Natl. Lab

Tainer, John
Scripps Research Institute

Terwilliger, Thomas
University of Illinois at Urbana-Champaign

Thorne, Robert
Department of Physics, Laboratory of Atomic and Solid State Physics, Cornell University

Tiede, David
Argonne National Laboratory
Chemistry Division, IL

Van Roey, Patrick
Wadsworth Center, NYS Dept of Health

Vekilov, Peter
Department of Chemical Engineering
University of Houston

Wang, Bi-Cheng
Biochemistry and Molecular Biology
Center for Metalloenzyme Studies
University of Georgia

Weitz, David
Department of Physics
Harvard University

Wery, Jean Pierre
Eli Lilly Corporation

Wiencek, John
Chemical and Biochemical Engineering
University of Iowa

Wilson, David
Molecular Biology & Genetics
Cornell University

Wilson, W. William
Physical Chemistry
Mississippi State University

Zukoski, Charles
Chemical and Biochemical Engineering
University of Illinois at Urbana-Champaign

References:

Books, Monographic Reviews and Proceedings:

A.McPherson, *Crystallization of Biological Macromolecules,* Cold Spring Harbor Laboratory Press (1999) 586 pp.

Macromolecular Crystallization in the Structural Genomics Era. Editor: A. McPherson. J. Structural Biology **142** (2003) 1-231. Special issue.

P.G. Vekilov and A.A. Chernov, *The Physics of Protein Crystallization*, in *Solid State Physics*, Editors: H. Ehrenreich and F. Spaepen, Academic Press: New York **57** (2002) 1-147.

Proceedings of the last four International Conferences on Crystallization of Biological Macromolecules (ICCBM):

ICCBM6, Japan: J. Crystal Growth **168** (1996) 1-323
ICCBM7, Spain: J. Crystal Growth **196** (1999) 185-710
ICCBM8, USA: J. Crystal Growth **232** (2001) 1-639
ICCBM9, Germany: Acta Crystallogr. **D58** (2002) 1511-1763

Original Publications:

A. George and W. W. Wilson, *Predicting protein crystallization form a dilute solution property.* Acta Crystallogr. **D50** (1994) 361-365.

C. Haas, J. Drenth, and W.W. Wilson, *Relation between the solubility of proteins in aqueous solutions and the second virial coefficient of the solution.* J. Phys. Chem. **B 103** (1999) 2808-2811.

P. M. Tessier, A. M. Lenhoff and S. I. Sandler, *Rapid measurement of protein osmotic second virial coefficients by self-interaction chromatography*. Biophys. J. **82** (2002) 1620-1631.

P. M. Tessier, H. R. Johnson, R. Pazhianur, B. W. Berger, J. L. Prentice, B. J. Bahnson, S. I. Sandler and A. M. Lenhoff, *Predictive crystallization of ribonuclease A via rapid screening of osmotic second virial coefficients.* Proteins: Structure, Function and Genetics **50** (2003) 303-311.

C. J. Hitscherich, J. Kaplan, M. Allaman, J. Wiencek, and P. J. Loll, *Static light scattering studies of OmpF porin: implications for integral membrane protein crystallization.* Protein Sci. **9** (2000) 1559-1566.

C. J. Hitscherich, V. Aseyev, J. Wiencek, and P. J. Loll, *Effects of PEG on detergent micelles: implications for the crystallization of integral membrane proteins.* Acta Crystallogr. **D57** (2001) 1020-1029.

N. Asherie, A. Lomakin, and G.B. Benedek, *Phase diagram of colloidal solutions.* Phys. Rev. Lett. **77** (1996) 4832-4835.

A. Lomakin, N. Asherie, and G. Benedek, *Aelotopic interactions of globular proteins.* Proc. Natl. Acad. Sci. USA **96** (1999) 9465-9468.

O. Annunziata, N. Asherie, A. Lomakin, J. Pande, O. Ogun, G. Benedek, *Effect of polyethylene glycol on liquid-liquid phase separation in aqueous protein solution*. Proc. Nat. Acad. Sci. **99** (2002) 14165-14170.

E. Grelet and S. Fraden, *What is the origin of chirality in the cholesteric phase of virus suspensions?* Phys. Rev. Lett. **90** (2003) Art. 198302.

J. Bloustine, V. Berejnov, and S. Fraden, *Measurements of Protein-Protein Interactions by Size Exclusion Chromatography.* Biophys. J. **85** (2003) In press (October).

D.F. Rosenbaum, P.C. Zamora, and C.F. Zukoski, *Phase behavior of small attractive colloid particles.* Phys. Rev. Lett. **76** (1996) 150-153.

S.-T. Yau and P.G. Vekilov, *Quasi-planar nucleus structure in apoferritin crystallization.* Nature **406** (2000) 494-497.

A. J. Malkin, T. A. Land, Yu. G. Kuznetsov, A. McPherson, J. J. DeYoreo, *Investigation of virus crystal growth mechanism by in situ atomic force microscopy.* Phys. Rev. Lett. **75** (1995) 2778-2781.

T. A. Land, A. J. Malkin, Y. G. Kuznetsov, A. McPherson and J. J. DeYoreo, *Mechanisms of Protein Crystal Growth: An Atomic Force Microscopy Study of Canavalin Crystallization* Phys. Rev. Lett. **75** (1995) 2774-2777.

A. J. Malkin and A. McPherson, *Novel mechanisms for defect formation and surface molecular processes in virus crystallization.* J. Phys. Chem. **B 106** (2002) 6718-6722.

S.-T. Yau, B.R. Thomas, and P.G. Vekilov, *Molecular mechanisms of crystallization and defect formation.* Phys. Rev. Lett. **85** (2000) 353-356.

A.A. Chernov, *Crystals built of biological macromolecules.* Physics Reports **288** (1997) 61-75.

B.R. Thomas, A.A. Chernov, P.G. Vekilov, and D.C. Carter, *Distribution coefficients of protein impurities in ferritin and lysozyme crystals. Self-purification in microgravity.* J. Crystal Growth. **211** (2000) 149-156.

C. L. Caylor, I. Dobrianov, S. G. Lemay, C. Kimmer, S. Kriminski, K. D. Finkelstein, W. Zipfel, B. R. Thomas, A. A. Chernov, and R. E. Thorne, *Macromolecular Impurities and Disorder in Protein Crystals*. Proteins: Structure, Function, and Genetics **36** (1999) 270-281.

L. J. DeLucas, T. L. Bray, L. Nagy, D. McCombs, N. Chernov, D. Hamrick, L. Cosenza, A. Belgovskiy, B. Stoops, and A. Chait, *Efficient Protein Crystallization*. J. Structural Biol. **142** (2003) 188-206

I. Dobrianov, K. D. Finkelstein, S. G. Lemay, and R. E. Thorne, *X-ray Topographic Studies of Protein Crystal Perfection and Growth.* Acta Crystallogr. **D54** (1998) 922-937.

C. L. Caylor, I. Dobrianov, C. Kimmer, W. Zipfel, and R. E. Thorne, *Two-Photon Fluorescence Imaging of Impurity Distributions in Protein Crystals*. Phys. Rev. **E59** (1999) R3831-R3834.

G.E.O. Borgstahl, A. Vaherdi-Faridi, J. Lovelace, H.D. Bellamy, and E.H. Snell, *A test for macromolecular crystallization in microgravity: large well ordered insulin crystals.* Acta Crystallogr. **D57** (2001) 1204-1207.

Z.W. Hu, B. Lai, Y.S. Chu, Z. Cai, D.C. Manchini, B.R. Thomas, and A.A. Chernov, *Phase sensitive x-ray diffraction imaging of defects in biological macromolecular crystals.* Phys. Rev. Lett. **87** (2001) 1481-1484.

Z.W. Hu, B.R. Thomas, and A.A. Chernov, *Laboratory multiple crystal x-ray topography and reciprocal space mapping of protein crystals: influence of impurities on crystal perfection.* Acta Crystallogr. **D57** (2001) 840-846.

I. Dobrianov, S. Kriminski, C. L. Caylor, S. G. Lemay, A. Kisselev, K. D. Finkelstein, and R. E. Thorne, *Dynamic Response of Tetragonal Lysozyme Crystals to Changes in Relative Humidity: Implications for Post-Growth Crystal Treatments*, Acta Crystallogr. **D57** (2001) 61-68.

S. Kriminski, C. L. Caylor, M. C. Nonato, K. D. Finkelstein and R. E. Thorne, *Flash Cooling and Annealing of Protein Crystals*. Acta Crystallogr. **D58** (2002) 459-471.

S. Kriminski, M. Kazmierczak and R. E. Thorne, *Heat Transfer from Protein Crystals: Implications for Flash Cooling and X-ray Beam Heating*. Acta Crystallogr. **D59** (2003) 697-708.

A. Vahedi-Faridi, J. lovelace, H. D. Bellamy, E. H. Snell, and G. E. O. Borgstahl, *Physical and structural studies on the cryocooling of insulin crystals.* Acta Crystallogr. **D59** (2003) In press.